# THE COLLECTION AND COMPOSITION OF THE UTERINE FLUID OF THE HEN

by

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## INTRODUCTION AND PURPOSE

For many years the domestic fowl has been the subject of scientific research, and the phenomenon of egg formation has been of great interest scientifically and economically. In recent years, this experiment station has undertaken an extensive study of the physiology of egg formation, and during this study a knowledge of the composition of the uterine fluid has become a necessary step in investigating the formation of the outer thin white of the laid egg. It has been known since the work of Pearl and Curtis (1) in 1912 that 40 to 50 percent of the weight of the egg white is added during the time the egg is in the uterus--hence after the formation of the shell membranes. The yolk, in passing down the oviduct, is surrounded by thick gelatinous albumen laid down by the glands of the magnum (the albumen-secreting portion of the oviduct). When the egg reaches the isthmus, loosely-fitting membranes are secreted to enclose the yolk and viscous white. From this point the egg passes to the uterus, where it lies for about 20 hours before being laid. During the first 6 or 7 hours in the uterus, a fluid passes through the shell membranes, causing the egg to

gain in weight by about 12 grams, and the membranes to become taut about the now plump egg. It is known also (1, 2, 3, 4) that after this fluid has entered the egg, the outer layer of thin albumen is present. Pearl and Curtis (1) reported this outer layer of thin white to be formed during these first few hours, and the noted gain in weight to be due to the infiltration of a fluid secreted by the uterus. This secretion may in turn be thought of as contributing to the formation of the outer thin white. None of the available literature has given any definite information as to the character of the secretion; therefore, as part of a comprehensive study of egg formation, it was thought advisable to examine the uterine fluid and to subject it to chemical analysis. The osmotic pressure of this substance should also be of interest, as its addition to the egg is by virtue of its diffusion through the shell membranes.

In a number of studies of egg formation, it is desirable to be able to reproduce <u>in vitro</u> the conditions in the uterus. In order to do this satisfactorily, the composition of the uterine fluid must be known. It is interesting to note also that, because this fluid contributes approximately one-half the total volume of the egg white, it is of importance in

determining the quality of the laid egg. In experimental work of this type, it is desirable to distinguish between the secretion and the fluid actually found in contact with the egg, since the fluid found in the uterus may hardly be considered the true uterine secretion, and the analysis of this fluid does not of itself give a reliable index to the nature of the secretion. The egg contents are enclosed only in the pliable shell membranes, through which the ions and molecules may diffuse from both sides. The liquid found in the uterus does not necessarily have the same composition as the uterine secretion, but is probably a complex mixture resulting from the interchange of ions between the egg and the secretion. Any ions which have diffused from the secretion into the egg will be absent from the fluid. These considerations indicate that studies involving the outer thin white must include a knowledge of the uterine fluid, for purposes of laboratory experiments using an artificial uterine fluid; but some other means of determining the character of the actual secretion must be devised.

The author's part in this major project was to collect samples of the fluid found in contact with the egg and to subject it to a more or less detailed analytical procedure.

## REVIEW OF IMPORTANT LITERATURE

The uterine secretion receives passing mention in the literature dealing with egg formation, and is almost invariably described as a thin albumenous solution which becomes the outer thin white through its addition to the membranous egg. Pearl and Curtis (1) in 1912 published a paper dealing with their work in regard to egg formation. Their work showed the increase of about 12 grams in the total weight of the egg white during the period in the uterus. They observed that the thin white was formed after the shell membranes were completed, and suggested that the formation was due to the infiltration of an albumenous solution from the uterus. This statement drew criticism from Hansen (2), who believed that the shell membranes must be assumed to be impermeable to albumen. The present author, however, has conducted a series of experiments to be described later, each one of which indicated that the shell membranes are permeable to albumen. Hansen agreed that the increase in size and weight while the egg was in the uterus lent support to the hypothesis of Pearl and Curtis, but asserted that the fluid which entered must be a salt solution instead of a protein solution, and presented figures showing an

increase in the ash content of the egg white during this period. On the other hand, Pearl and Curtis (1) found 0.22 percent nitrogen in a sample of uterine fluid removed from a freshly killed hen, and exhibited this finding as further support of their viewpoint.

McNally (4) confirmed the findings of Pearl and Curtis, and reported that the increased protein content was due to the addition of ovoglobulins. However, Hughes and Scott (5) reported that the apparent increase in globulin found by McNally appears in the inner layer of white instead of near the shell membranes, as would be the case if it were a part of the uterine secretion. Scott, Hughes, and Warren (6) stated that there is a variation in size among eggs of a clutch; that is, each succeeding egg of a clutch is usually smaller than the preceding one. Thus in the average instance, when an egg is intercepted and its protein content compared with that of the previously laid egg, it is found to contain less nitrogen. But this same egg, if allowed to go to completion, would be smaller than its predecessor, hence still would have less nitrogen than was found in the preceding egg. Correction factors obtained from this work and applied to previous findings indicate that no protein is added to the egg during its stay in

the uterus.

Richardson (7), in a histological study of the oviduct, reported the presence of secretory glands in the uterus, and made this statement:

"The primary function of the uterine glands in secreting the thin fluid albumen is confirmed by most authors and need not be further discussed beyond mentioning that its addition to the egg-white must necessarily be confined to the period during which the shell matrix is still elastic and pliable."

The presence of secretory glands in the uterus is noted also by Froböse (8).

It is apparent from the above that the composition of the uterine fluid has been of interest for at least 25 years, and while its possible contents have been indicated by observations of the egg, its actual composition has not been determined by direct analysis.

## MATERIAL AND METHODS

The hens used as a source of uterine fluid were from the Kansas State College flock, and were not selected according to breed or clutch size. There were two reasons for this lack of selection; first, the samples obtainable at any given time were of such small volume and so difficultly collected that to restrict the selection was merely to increase difficulties; and second, there is nothing to indicate that this type of study of egg formation has need to be restricted to any particular type of hen.

The removal of the fluid from the uterus in an uncontaminated state presented some difficulties, and the first attempts were not entirely successful. The hens were trapnested hourly, and at the end of six hours after laying, those hens whose uteri contained membranous eggs were used. The presence of the eggs is easily verified by palpation. The membranous egg in each instance was removed by digital manipulation, and into the uterus was then inserted a collapsed rubber balloon, attached to the end of a length of rubber tubing, which in turn was bound parallel to a soft rubber

catheter. The exposed ends of the catheter and the rubber tubing were attached to a glass vial and a rubber bulb respectively. Pressure was applied by means of the bulb to distend the uterine walls to the proportions attained in the presence of an egg, and the fluid drained through the catheter into the vial. As may be imagined, this process was quite cumbersome, and there was often some bleeding as a result of abrasion of the vaginal tract. In an attempt to circumvent this difficulty, the following simplified procedure was employed. About six hours after laying, the presence of membranous eggs was verified, and the birds were catheterized by inserting a rubber tube into the uterus by way of the vagina. By placing the tip of the tube ventral to the egg, the fluid present in the lumen was drained into a small vial and preserved in a glass-stoppered weighing bottle. There were some mechanical difficulties encountered in the collection of samples in this manner, but fluid in quantities up to 3 cc. were obtained with comparative ease. Since the excretory organs as well as the vagina empty into the cloaca, any method of catheterization of the vagina and subsequent draining of the fluid from the uterus is accompanied by the danger of contamination from excreta, both by

their being carried to the uterus on the catheter, and by their entering the catheter on its withdrawal. The first-described and somewhat elaborate procedure was employed as a result of the belief that the secretion was absorbed by the egg directly from the uterine wall, and that in order to allow the fluid to accumulate in the lumen, the egg must be replaced by a relatively impermeable membrane; but when the second, simplified method was used, it was learned that the secretion does not pass directly from the uterine wall through the shell membranes, but that it accumulates in the ventral pocket formed by the weight of the egg. Contamination by excreta was guarded against by the use of cotton plugs, but the efficiency of these plugs became questionable when uric acid in quantities of from 18 to 25 milligrams per 100 cc. was found. On obtaining the fluid by incision of the uterine wall and avoiding the cloaca entirely, it was learned that uric acid was present to the extent of only about 4.6 milligrams per 100 cc. This concentration is approximately the same as that found in normal chicken blood; for this reason, all analyses were checked by the use of samples obtained by surgery. By this procedure, the analyses are believed to be uninfluenced by contaminants from

the excretory system.

This final method consisted of verifying the presence of a membranous egg in the uterus, then strapping the bird to an operating table and injecting 3/4 grain of Nembutal intravenously. After the onset of complete anaesthesia, an incision was made in the abdominal wall, through which the uterus was reached. A small section, or wedge, of this wall was isolated by means of clamps to prevent bleeding into the uterus, and an incision made, through which the fluid was removed by means of a rubber catheter.

Recognized methods of analysis were used throughout, as follows:

The percentages of total solids and of ash were determined by the methods described by Hawk and Bergeim (9, pages 198 and 199).

Total alkali was determined on the ash by Barnett's method (10), and the potassium was estimated by the method of Cameron and Failyer (11). The sodium was calculated by difference.

Chlorides in the ash were determined by the method of Whitehorn (12, page 150).

The calcium determinations were made on aliquots of the original sample by the colorimetric method of

Roe and Kahn (12, page 156).

The phosphorus analyses were made by the Fiske-Subbarow method, as modified by Koch (12, page 150).

The determinations of carbon dioxide were made according to the Van-Slyke-Neill manometric method, on samples collected under toluene (12, page 162).

Protein nitrogen was estimated by the direct nesslerization method of Koch and McMeekin (12, page 119).

The Folin-Wu method was used for determining reducing substances (12, page 141).

The pH of samples collected under toluene was determined by means of the quinhydrone electrode.

The method of Denis (9, page 462) was used for the determination of magnesium.

Sulfates were estimated by the direct titration method of Sheen and Kahler (13).

## SUMMARY OF RESULTS

The results of the analyses of some 50 samples are given in Table 1. Due to the small size of the samples, no one specimen could be subjected to a detailed analysis, but the average figures in the table refer to data from at least six samples in each case. In the instances of protein nitrogen, calcium, solids, ash, chlorides and pH, the data represents analysis of 12 to 24 samples for each constituent. In the determinations of magnesium, reducing substances, and phosphorus, the small amounts found did not indicate the need of a large number of analyses.

TABLE I

Composition of the Uterine Fluid of the Domestic Fowl

| | Max. % | Min. % | Mean % |
|---|---|---|---|
| Solids | 1.75 | 1.06 | 1.40 |
| Ash | 0.94 | 0.67 | 0.90 |
| Protein Nitrogen | 0.08 | 0.03 | 0.06 |
| Sodium ($Na^+$) | 0.284 | 0.131 | 0.256 |
| Potassium ($K^+$) | 0.111 | 0.055 | 0.098 |
| Calcium ($Ca^{++}$) | 0.028 | 0.012 | 0.019 |
| Magnesium ($Mg^{++}$) | 0.002 | 0.000 | 0.001 |
| Chlorides ($Cl^-$) | 0.296 | 0.230 | 0.255 |
| Bicarbonates ($HCO_3^-$) | 0.353 | 0.339 | 0.345 |
| Sulfates ($SO_4^{--}$) | | | None |
| Phosphates ($PO_4^{---}$) | | | None |
| Reducing Substances | | | None |

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pH 7.74 to 7.90

## DISCUSSION

The apparently large loss in weight on ignition (Table 1) is not to be considered entirely as organic matter. While it does include the protein and other traces of organic matter, the loss is augmented by the decomposition of those salts present as bicarbonates, which are not stable at ashing temperatures.

A notable point to be observed from the analysis of the uterine fluid is that it is not an albumenous solution such as that of which the thin white is composed. The small amount of protein present may well be considered a contaminant, as the shell membranes are permeable to protein. Hansen (2) did not believe the shell membranes to be permeable to protein, but the present author has repeatedly and without exception observed diffusion through these membranes in a number of experiments.

In making the tests for permeability, sections of fresh membranes were carefully washed in Ringer's solution and sealed onto the ends of hollow glass tubes containing albumen, and these tubes immersed in Ringer's solution at 38° C. At the end of 3 hours, sufficient protein had diffused out into the solution that its

presence could be verified by the addition of 10 percent trichloroacetic acid, in which case a flocculent precipitate resulted. To further substantiate these results, carefully washed membranous eggs were placed in warm Ringer's solution, and at the end of 3 to 4 hours, when the egg had become plump and the membranes normally taut, the presence of protein in the surrounding medium was again verified by the formation of a flocculent precipitate on the addition of 10 percent trichloroacetic acid. The question then arose as to whether the presence of the protein in the surrounding medium might be due either to lack of thoroughness in washing or to the solution of the membranes; therefore, about a dozen segments of membranes were washed in the same manner as were the eggs and placed in test tubes containing Ringer's solution. At the end of 24 hours the addition of 10 percent trichloroacetic acid failed to produce visible flocculation.

Nathusius (14) reported the shell membranes to consist of a network of microscopic fibers, and it is difficult to conceive of a network composed of fibers so large as to be visible under the microscope, which would be impermeable to particles of such small size

as protein molecules.

Attempts were made to study the colloidal osmotic pressure of this uterine fluid. A modification of the Krogh-Wakazawa osmometer was used, as described by Dubach and Hill (15). Due, however, to faulty construction of the apparatus, it was not found to be practically possible to prevent leakage, and after spending a considerable time in polishing the surfaces of the device, the author temporarily discontinued the studies. Since the composition of the fluid is now known, it is a fairly safe assumption that little or no substances are in the liquid to give it an appreciable colloidal osmotic pressure, the protein nitrogen content being only 0.06 percent. It should be pointed out that even though the shell membranes are permeable to proteins, there is such a great difference in particle size between the crystalloids and the proteins present, that the egg white is capable of exerting considerable osmotic pressure during the short time required for the addition of the fluid. The crystalloids may be considered as reaching a condition of comparative equilibrium, a state unattainable by the proteins in this brief time.

It is a well-known principle that when two solu-

tions of different composition are separated from each other by an inert permeable membrane, any net diffusion of a constituent will take place from the region of high concentration to the region of low concentration. If enough time elapses, a condition of equilibrium will be established, with all substances having equal activities on the two sides of the membrane. When molecules such as proteins are present, adsorption may prevent some of the ions from active diffusion, and the actual concentration will not be quite the same as the effective concentration, or "activity".

Since the amount of protein nitrogen in the egg white next the shell membranes is roughly 1 percent*, while it is only 0.06 percent in the uterine fluid (Table 1), it is highly improbable that proteins can diffuse into the egg, thereby increasing the protein content of the white. On the contrary, the white must slowly lose protein to the uterine fluid. This accounts not only for the presence of the small amount of protein in the uterine fluid, but may explain also the source of at least a part of the protein found in the eggshell.

The small and variable amounts of magnesium found, ranging from none at all to 2 milligrams per 100 cc.

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*Unpublished laboratory data.

indicate that it is a contaminant from the egg.

Calcium concentrations were notably variable. The calcium which goes to form the shell is not necessarily a part of the fluid which has been analyzed. In fact, the results indicate that about 10 liters of fluid would be required to furnish enough calcium to form the shell if it were obtained in this manner. Richardson's (7) histological findings indicate that the shell is deposited by the secreting glands directly onto the shell membranes. Regardless of the mechanism of shell deposition, one may expect wide variations in the amounts of calcium found in the fluid surrounding the egg, due to the presence of unattached shell particles.

It has been previously pointed out that the fluid which the author has analyzed is simply the fluid that is found in contact with the uterine egg, and is not necessarily the true uterine secretion, but a complex mixture resulting from the interchange of ions across the shell membranes between the egg and the uterine secretion. Beadle and Conrad (16) have approximated the composition of the true secretion by the analysis of membranous eggs removed prior to outer thin white formation. The results of each of these analyses were compared with those obtained from the preceding egg

laid by the same bird, after applying correction factors for variations in egg size, as described previously. After this correction, if the laid egg contains more of any substance than the uterine egg, this increase probably has come from the uterine secretion. Any substance which is present in lesser amounts in the laid egg than in the uterine egg probably has diffused out into the uterine fluid due to a smaller concentration of that substance in the uterine secretion; while any ion which is present in greater amount in the laid egg than in the uterine egg must have diffused from the uterine fluid into the egg because of a greater concentration of that ion in the uterine secretion. On the basis of these comparative analyses, the amount of each substance added to the egg was calculated and expressed in concentration terms, by dividing the increment of each substance by the total volume increase of the egg.

The figures obtained in this manner give the character of the solution added to the egg during the time it is in the uterus, while the figures the author has obtained by direct analysis give the composition of the fluid found in contact with the egg.

As shown in Table 2, the liquid added to the egg

TABLE 2

Comparative Ion Concentrations in Uterine Eggs and Laid Eggs

Results in Mgms./100 cc.

| Ion | Uterine Egg | | | Laid Egg | | | Concentration in Increment | | |
|---|---|---|---|---|---|---|---|---|---|
| | Max. | Min. | Ave. | Max. | Min. | Ave. | Max. | Min. | Ave. |
| $Na^{+}$ | 319 | 277 | 296 | 223 | 182 | 199 | 142 | 15 | 71.5 |
| $K^{+}$ | 76 | 51 | 58 | 162 | 101 | 125 | 317 | 131 | 214 |
| $Ca^{++}$ | 63.9 | 38 | 55 | 72.4 | 21.4 | 32 | 102 | -35 | 0.4 |
| $Mg^{++}$ | 28.4 | 18.8 | 24 | 20.6 | 9.7 | 13 | 14.4 | -11.7 | -0.08 |
| $Cl^{-}$ | 323 | 280 | 299 | 188 | 140 | 162 | 124 | -21 | 43 |
| $HCO_3^{-}$ | | | | | | | | | 512* |

* Calculated from excess alkali

differs considerably from the fluid found in the uterus.

An outstanding characteristic of the uterine secretion is its unusually high potassium concentration. This concentration of 214 mgms. per 100 cc. (Table 2) is all the more striking when one considers that it is about 10 times the potassium content of normal blood serum, which has been reported to be about 22 mgms. per 100 cc. (17).

Although this potassium content of 214 mgms. per 100 cc. is extremely high, there are several biological fluids which have been reported as containing considerably larger amounts of potassium than is found in normal blood serum. Conklin, McCarthy, Thompson, and Pugsley (18) have reported that bovine amniotic fluid contains, on an average, 62.6 mgms. potassium per 100 cc., while Howe (19) has found the potassium in cow's milk to be about 150 mgms. per 100 cc. Egg white has been reported by Grossfeld and Walter (20) to contain 153 mgms. per 100 cc. The close relation which these three fluids bear to young rapidly growing tissues calls attention to the fact that various workers have pointed out that high potassium content is a characteristic of rapidly proliferating tissue (21, 22, 23). Mention is also made in the literature of the apparently high potassium demands made by the embryo and fetus (23, 24).

# CONCLUSION

The uterine secretion of the domestic fowl is not an albumenous solution such as that which makes up the thin white of the laid egg. It is essentially a mineral solution, consisting of sodium, calcium, and potassium, present as chlorides and bicarbonates.

Efforts to determine the colloidal osmotic pressure of the uterine fluid were unsuccessful, due to faulty apparatus, but it follows from the lack of protein that the colloidal osmotic pressure is very low.

The potassium content of the secretion is quite high, being about 10 times that found in normal blood serum. The possible significance of this high potassium level in supplying the demands of the embryo is indicated by reports of other workers, who have found high potassium levels in related fluids. Some of these reports indicate that the young animal is surprisingly high in its potassium demands.

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